RBMO

REVIEW

New strategies of ovarian stimulation based on the concept of ovarian follicular waves: From conventional to random and double stimulation

BIOGRAPHY

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KEY MESSAGE

New ovarian stimulation approaches, such as luteal/random start and double stimulation, are useful for meeting the double demands of optimizing flexibility in ovarian stimulation and improving IVF outcomes. Such protocols may be applicable for fertility preservation in cancer patients and for increasing oocyte yield in poor responders.

ABSTRACT

The theory of a multicyclic development of follicles during the menstrual cycle prompted new approaches to ovarian stimulation, such as starting gonadotrophins for ovarian stimulation at any time during the menstrual cycle or using double stimulation during it, with stimulation in both the follicular and luteal phases. Because of the asynchrony between endometrial receptivity and embryo development with a 'non-conventional start' stimulation, all the oocytes/embryos are generally cryopreserved and transferred subsequently. This deferred transfer policy is currently possible given the advances in vitrification techniques, with success rates comparable to those following transfer with 'fresh' embryos. New stimulation approaches, together with advanced cryopreservation techniques, allow for a total 'disarticulation' between the time of the menstrual cycle, ovarian stimulation start and embryo transfer. This new approach to ovarian stimulation is particularly useful for women seeking fertility preservation, especially where a shortened time to starting cancer treatment is desirable. Also, poor responders could benefit from the new stimulation protocols by continuing ovarian stimulation after the first oocyte retrieval, thereby obtaining more oocytes or embryos compared with the conventional approach.

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KEYWORDS
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INTRODUCTION

An IVF treatment cycle consists of various stages: ovarian stimulation, mostly with gonadotrophins; use of gonadotrophin-releasing hormone (GnRH) agonists or antagonists for pituitary suppression, thereby avoiding premature ovulation; ovulation trigger for final oocyte maturation; timed oocyte retrieval; IVF of gametes; embryo culture and finally embryo transfer. Most current IVF programmes involve a fresh embryo transfer policy, with spare embryos frozen for subsequent use, either when the fresh cycle fails to result in a pregnancy or when couples want another child following a successful cycle. Ovarian stimulation is a fundamental part of IVF programmes, in which exogenous gonadotrophins are used to achieve supraphysiological levels during the period of follicular recruitment to override the process of dominant follicle selection and enable multiple follicular recruitment (Macklon, 2006). FSH is usually administered in the early follicular phase. The mean duration of stimulation is 11–12 days and in this period there is progressive growth of antral follicles, steroid production by the ovaries and endometrial proliferation. The object of stimulation is recruitment of multiple follicles in order to have many oocytes and increase the chances of pregnancy in IVF.

Recent research has suggested that recruitable antral follicles are continuously present in the ovaries during the menstrual cycle, and ultrasonographic studies have demonstrated that multiple cohorts or 'waves' of 2–5 mm follicles are recruited continuously during a menstrual cycle (*Baerwald, 2003*a). A wave is a synchronous growth of follicles that have a similar diameter. Waves of follicle development have been documented in healthy women using ultrasonography (*Baerwald, 2003*b).

It has been proposed that there are two waves of follicular growth during the menstrual cycle. The first wave occurs in the follicular phase and a second wave in the luteal phase (FIGURE 1). Most women appear to exhibit two waves, with a minority exhibiting three waves. Women with three waves may have a longer menstrual cycle compared to women with two waves (*Baerwald, 2003*b). The inhibin B produced from the recruited cohort inhibits FSH secretion during the mid-follicular phase. A second short peak of inhibin B is present after the LH surge, supporting the idea of a second wave of follicles during the luteal phase (*Frachimont, 1975*). The follicular wave that emerges in the early to mid-follicular phase is ovulatory, while the wave or waves emerging in the luteal phase are usually anovulatory. Follicular waves are also present in women during perimenopausal transition (*Hale, 2007*) and in women undergoing ovarian stimulation (*Bentov, 2010*). The follicular waves could be recruited by constantly high concentrations of FSH. This new knowledge about ovarian function and, in particular, the theory of a multicyclic development of follicles during the menstrual cycle, resulted in the prospect of new approaches to ovarian stimulation and, in particular, the random-start ovarian stimulation protocols, namely the administration of exogenous gonadotrophins randomly on any day of the monthly menstrual cycle. In parallel with these new theories in ovarian stimulation protocols, advances in vitrification of embryos and oocytes has allowed important progress to be made towards a new concept of a total 'disarticulation' between ovarian stimulation and embryo transfer.

As a consequence, new ovarian stimulation protocols can be proposed to those patients who could potentially benefit. These are useful particularly in cases of oncology patients seeking fertility preservation who may present at any stage during the menstrual cycle (*Cakmak and Rosen, 2013; von Wolff, 2009*). Adhering to the convention of initiating controlled ovarian stimulation at the beginning of the follicular phase may result in either significant delay of cancer treatments or foregoing fertility preservation because of time constraints. Of course, these new protocols may also be useful to women who want to preserve fertility for non-medical reasons, where double stimulation protocols might help with oocyte/embryo accumulation (*Moffat, 2014; Tsampras, 2017*). It has been suggested that double stimulation might serve as a useful strategy for poor ovarian response patients where accumulation of more oocytes in a short time could improve IVF outcomes (*Cardoso, 2017; Kuang, 2014; Liu, 2017; Ubaldi, 2016*).

This review article examines the evolution of new forms of ovarian stimulation due to expanding knowledge of ovarian physiology and discusses the implications of this strategy in clinical practice.

RANDOM- AND LUTEAL PHASE-START OVARIAN STIMULATION PROTOCOLS

The need to start ovarian stimulation on any day of the menstrual cycle initially occurred in an oncology setting. Fertility preservation for cancer patients has become essential in oncology care. Cancer treatment (especially alkylating agents such as cyclophosphamide or total body irradiation prior to stem cell transplantation) is often cytotoxic and may result in depletion of ovarian follicles with subsequent subfertility and ovarian failure (*Gracia, 2012; Letourneau, 2012*). Ovarian stimulation for embryo or oocyte cryopreservation is the preferred method of fertility preservation in post-pubertal girls and women with cancer and the only technique approved by the American Society for Reproductive Medicine (ASRM) (*Ethics Committee of American Society for Reproductive Medicine, 2013*). All other methods of fertility preservation, such as ovarian tissue cryopreservation, ovarian transplantation, in-vitro maturation of immature oocytes and the use of GnRH analogues are still considered investigational (*Ethics Committee of American Society for Reproductive Medicine, 2013*), although ovarian tissue cryopreservation is the only possibility when the cancer treatment cannot be postponed (Donnez, 2017). Traditionally, ovarian stimulation is initiated at the start of the follicular phase, but this method may require several weeks, depending on the patient's menstrual cycle phase at the time of the initial visit. The theory of multiple follicular waves and hence the possibility of random-start ovarian stimulation is an attractive approach for cancer patients. In cancer patients, there is no need to achieve synchrony between ovaries and endometrium as there is no fresh embryo transfer, thus allowing random-start stimulation protocols.

Luteal phase- and random-start protocols have also recently been applied outside the oncological setting, in normal and poor responders.

Luteal phase protocols initiate ovarian stimulation between days 15 and 20

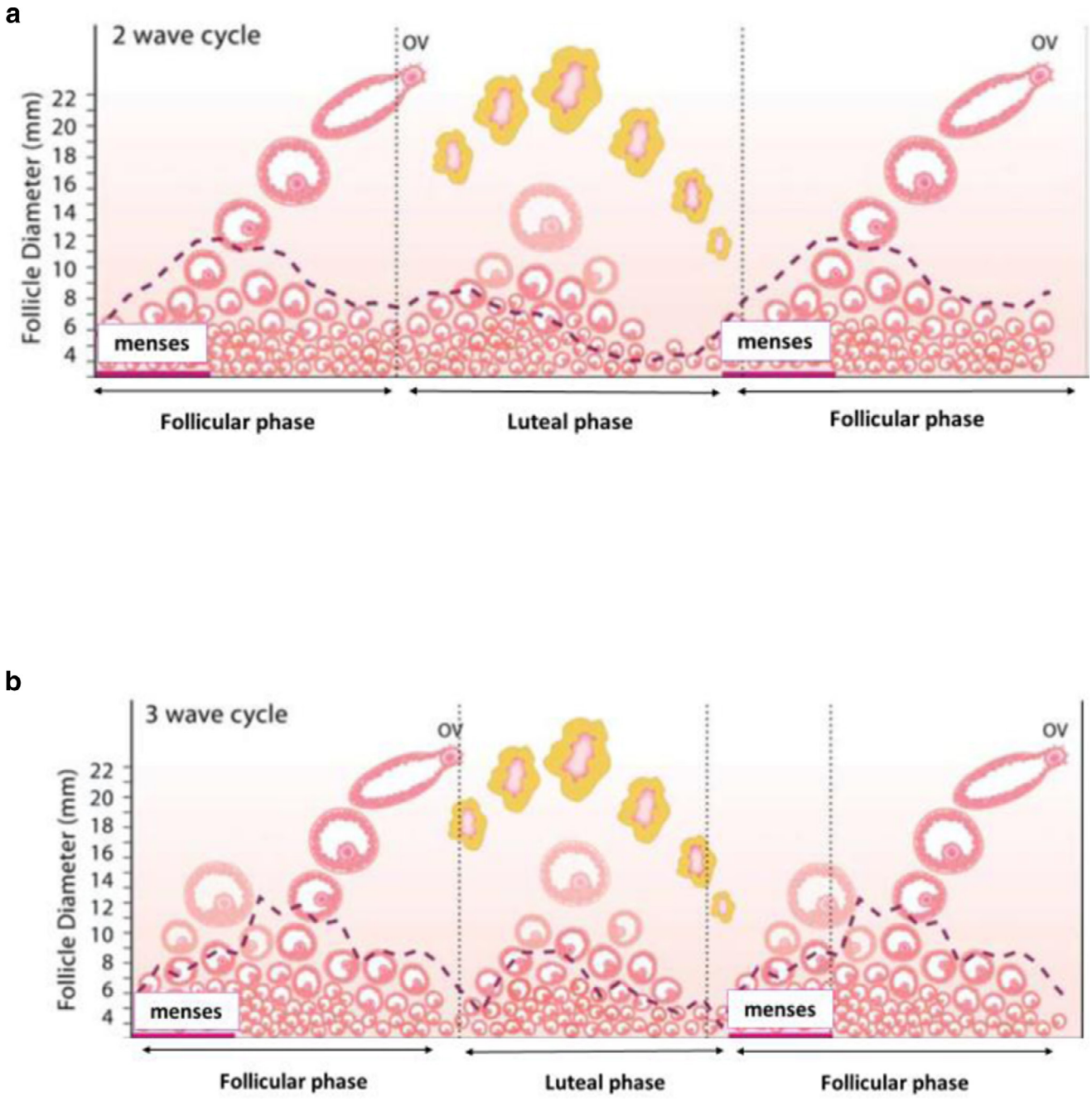

FIGURE 1 The wave theory of follicle recruitment suggests that two or more waves of antral follicles emerge during the ovarian cycle. In women with two follicular waves (a), an anovulatory wave emerged at the early luteal phase followed by emergence of the ovulatory wave during the early follicular phase. In women with three waves (b), an anovulatory wave emerged at the time of ovulation (ov), a second anovulatory wave emerged during the mid to late luteal phase and the ovulatory wave emerged in the early to mid-follicular phase. The dominant follicle develops in a minority of anovulatory waves. Follicle 'cyclic recruitment' is the process by which a single 'dominant' follicle is chosen from the recruited cohort or wave for preferential growth (modified with permission from *Baerwald et al., 2012*).

of the menstrual cycle (FIGURE 2). The largest study on luteal phase stimulation was performed by *Wang et al. (2016)* on normal responder patients. They compared three protocols: short agonist protocol, mild stimulation with clomiphene or letrozole followed by 225 IU of human menopausal gonadotrophin (HMG) and luteal stimulation with 225 IU of HMG. Implantation rates were identical with luteal stimulation (35.5%, $N = 727$) and mild stimulation (34.8%, $N = 830$), but significantly lower with the standard short agonist protocol (31.8%, $N = 1385$, $P = 0.012$). The same group demonstrated in a retrospective study that the luteal stimulation (clomiphene then HMG beginning at day 1 or 2 post-ovulation) was associated with a significantly higher number of oocytes collected and top-quality embryos (2.8 versus 2.0, $P < 0.05$ and 0.9 versus 0.4, $P < 0.05$, respectively) compared with conventional-start stimulation (clomiphene then HMG beginning at day 3 with a flexible antagonist) but also significantly longer ovarian stimulation and higher dose of HMG required. This study has some limitations: it is a

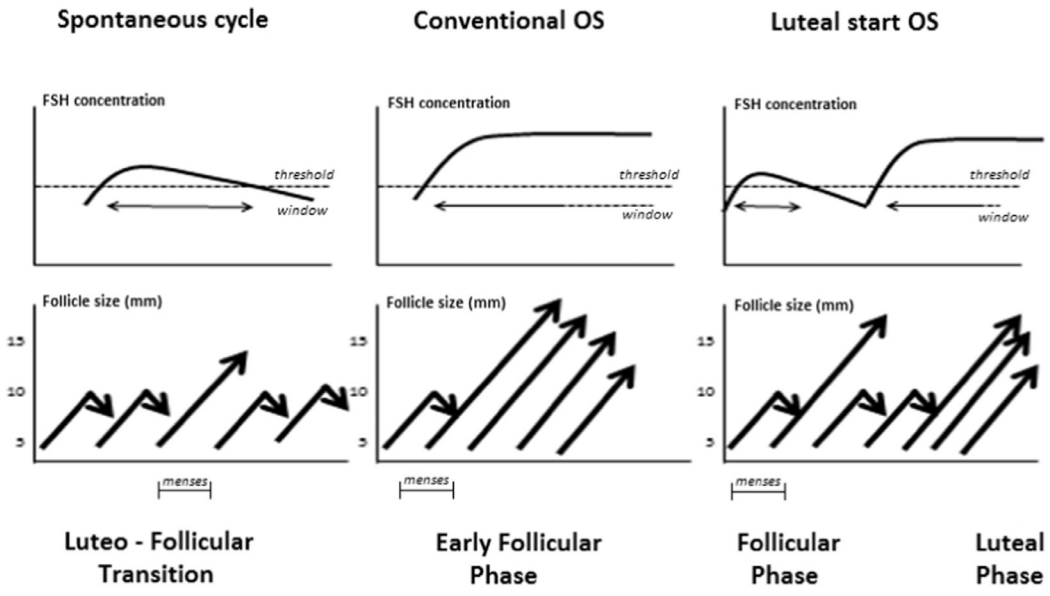

FIGURE 2 The FSH threshold and window in a spontaneous, conventional ovarian stimulation and luteal-start ovarian stimulation cycle. During the luteal phase a longer FSH window permits multiple follicular growth.

retrospective study and the number of retrieved oocytes (5.9–6.6) appears very low for normoresponder patients (*Li, 2016*).

To date few studies have been published reporting the efficacy of random-start protocols. However, preliminary results indicate that the number of total and metaphase II oocytes retrieved and fertilization rates are similar in early follicular and random-start protocols. Random-start ovarian stimulation results in a similar length of ovarian stimulation and in similar gonadotrophin use. 'Random-start' control ovarian stimulation protocols were reported to be successful in women with cancer who started stimulation on menstrual cycle days 11, 14 or 17 (*Sönmezer, 2011*). In three patients with a diagnosis of breast cancer requiring emergency fertility preservation in the late follicular or luteal phase of the menstrual cycle, random-start ovarian stimulation was commenced immediately on menstrual cycle days 11, 14 or 17 with use of letrozole 2.5 mg/day and recombinant FSH 150–300 IU/day (*Sönmezer, 2011*). In another study, in a 27-year-old woman with Hodgkin lymphoma, ovarian stimulation was performed with recombinant FSH 450 IU starting on day 11 of the menstrual cycle (*Ozkaya, 2012*). GnRH antagonist was administered to prevent ovulation in all four cases. Ovulation was triggered with either 250 mg of recombinant HCG, 10,000 IU of urinary HCG or 0.1 mg of leuprolide acetate. Nine to 31 oocytes were obtained, resulting in a good number of embryos to be frozen (*Ozkaya, 2012; Sönmezer, 2011*).

This approach resulted in a considerable number of oocytes and embryos. Also, the ovary with corpus luteum showed a similar number of dominant follicles compared with the patient's contralateral ovary on the day of the trigger (*Ozkaya, 2012*). Of course, the random-start ovarian stimulation can also be proposed in an IVF setting, for example in oocyte donors and in clinics that practise 'freeze all' almost routinely due to the use of clomiphene citrate to reduce the cost of attempts. In the study by *Qin and colleagues (2016)*, ovarian stimulation in infertile women prior to IVF was started independently of the day of the menstrual cycle. A total of 150 women were treated and ovarian stimulation was started in the early, late follicular or luteal phase. Flexibility in starting stimulation at any day of menstrual cycle stems from the fact that patients in China come from many different districts, in different phases of the menstrual cycle and wish to commence infertility therapy as soon as possible. There were no differences in the mean number of mature oocytes retrieved in the conventional group, late follicular phase group and luteal phase group (5.7 ± 3.6, 5.2 ± 3.7 and 5.2 ± 3.9, respectively). In the subsequent frozen embryo transfer cycles, the clinical pregnancy rates (41.5%, 45.5% and 38.9%), and implantation rates (30.7%, 30.2% and 27.1%) were similar in the three groups (*Qin, 2016*). At the moment it remains quite difficult to translate these preliminary studies to routine clinical practice because of the low number of patients, the heterogeneity of groups and the fact that they are retrospective studies.

DOUBLE OVARIAN STIMULATION (DOS) REGIMEN

The concept of continuous ovarian follicular growth, random start of ovarian stimulation and the disarticulation of stimulation and embryo transfer led to the possibility of performing consecutive ovarian stimulation cycles and oocyte retrieval procedures in order to increase the number of oocytes available (and possibly embryos) per patient in one given cycle.

DOS consists of two successive ovarian stimulations in the follicular and ensuing luteal phase with two oocyte retrievals at the end of both ovarian stimulations (FIGURE 3A).

The efficacy of a DOS during the follicular and luteal phase was reported for the first time a few years ago (Kuang, *2014*) (TABLE 1). Use of a second ovarian stimulation during the luteal phase was first applied in poor responder patients: for patients who respond poorly to

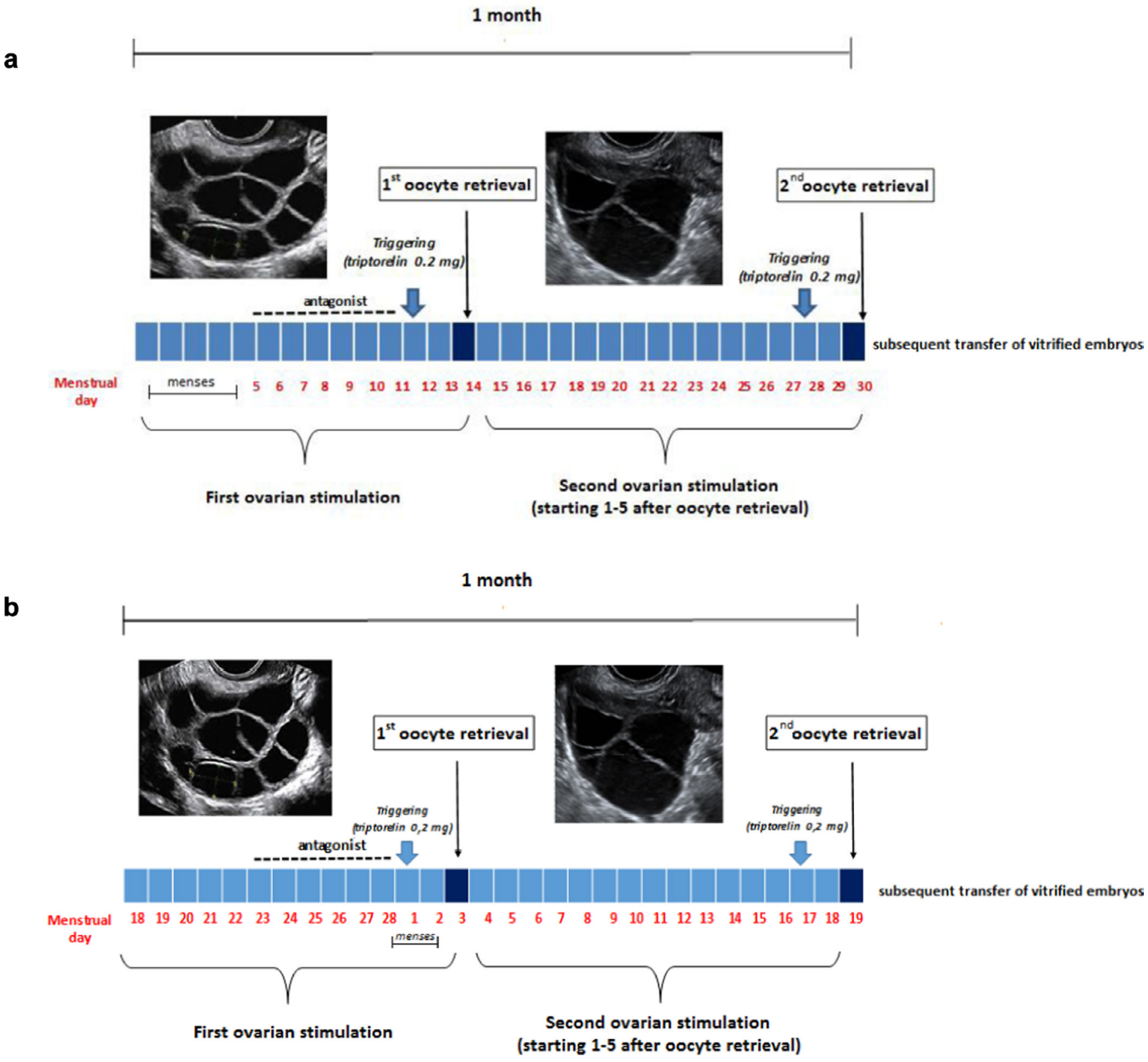

FIGURE 3 (a) Double stimulation regimens: two successive ovarian stimulations with two oocyte retrievals at the end of both ovarian stimulations are performed in less than 1 month. The first ovarian stimulation starts during the early follicular phase and the second can begin the day after the first oocyte retrieval. (b) Double randomly started ovarian stimulation (DoubleRandom-OS): two successive ovarian stimulations with two oocyte retrievals at the end of both ovarian stimulations are performed in approximately 25 days. The first ovarian stimulation can start randomly during the menstrual cycle (e.g. during the luteal phase), and the second can begin the day after the first oocyte retrieval. If ovarian stimulation starts during the luteal phase normally after some days of gonadotrophin therapy menstruation occur.

gonadotrophin stimulation various treatment protocols have been used as patient-friendly techniques to optimize the relationship between safety and success (*Loutradis, 2003; Ubaldi, 2005*) but the prognosis in these patients remains poor, with a live birth rate of 6.8–7.9% (*Ferraretti and Gianaroli, 2014; La Marca, 2015; Polyzos, 2014*).

Kuang et al. (2014) tested the possibilities of two ovarian stimulations during the follicular and luteal phase and showed good results in terms of more opportunities for retrieving oocytes. In these regimens the first stimulation started normally in the early follicular phase, while the second stimulation started on the first day after the first oocyte retrieval on which two or more antral follicles were identified. The trial was conducted in 38 patients who all fulfilled the Bologna criteria for poor responders, based on prior ovarian stimulation outcome, baseline hormonal levels and age. The first and second stimulations used two different regimens as follows: the first stimulation was conducted using a combination of clomiphene citrate 25 mg per day starting on day 3 of the cycle until the triggering of ovulation, letrozole 2.5 mg per day starting on day 3 for a total of 4 days and HMG 150 IU every other day, starting on day 6 until the triggering of ovulation. The second stimulation was

TABLE 1 SUMMARY OF RESULTS OF STUDIES ON DOUBLE OVARIAN STIMULATION.

Author	Type of study	Patients	*n*		Protocol	Length of stimulation (days)	FSH/HMG consumption (IU)	Number of oocytes (*n*)	Number of embryos (*n*)	Pregnancy rate (%)
Kuang et al., 2014	Pilot study	Poor responders	38	First ovarian stimulation	Clomiphene 25 mg/day+ letrozole 2.5 mg daily + HMG + GnRH antagonist GnRH agonist triggering	10.2 ± 2.4	326 ± 248	1.7 ± 1	1 ± 1	48 (11/23)
			30	Second ovarian stimulation	Letrozole + HMG 225 IU + MPA. GnRH agonist triggering	10.8 ± 3.1	1802 ± 712	3.5 ± 3.2	2 ± 2.4	71 (5/7)
Ubaldi et al., 2016	Prospective study	Poor responders	42	First ovarian stimulation	FSH 300 IU + LH 75 IU + flexible GnRH antagonist. GnRH agonist triggering	9.6 ± 2.4	na	5.1 ± 3.4	2.3 ± 1.7	85.7 (6/7)[a]
			42	Second ovarian stimulation	FSH 300 IU + LH 75 IU + flexible GnRH antagonist. GnRH agonist triggering	10.3 ± 2.5	na	5.7 ± 3.3	3.2 ± 2.3	75 (6/8)[a]
Cardoso et al., 2017	Prospective study	Poor responders	13	Two consecutive ovarian stimulation cycles	FSH 225 IU + HMG 75 IU + fixed GnRH antagonist. GnRH agonist triggering	na	na	11.7 (range 1–28)	na	na
Liu et al., 2017	Retrospective study	Poor responders	116	First ovarian stimulation	Short GnRH agonist, GnRH antagonist protocol, mild stimulation with progestin down-regulation. HCG triggering	8.2 ± 3.5	1882 ± 958	2.33 ± 1.99	1.66 ± 1.47	25
			116	Second ovarian stimulation	HMG 225 IU. HCG triggering	7.5 ± 3.3	1728 ± 937	3.5 ± 3.55	2.4 ± 2.7	20.59
Tsampras et al., 2017	Retrospective	Oncological patients	10	First ovarian stimulation	HMG 150–450 IU + GnRH antagonist. HCG triggering	11.6 (range 9–14)	na	8.1 (range 1–13)	na	na
			10	Second ovarian stimulation	HMG 150–450 IU + GnRH antagonist. HCG triggering	11.6 (range 10–17)	na	8.2 (range 1–19)	na	na

Data are mean ± SD unless otherwise indicated.

GnRH = gonadotrophin-releasing hormone; HCG = human chorionic gonadotrophin; HMG = human menopausal gonadotrophin; MPA = medroxyprogesterone acetate; na = not available.

[a] per euploid blastocyst transfer.

started after the first oocyte retrieval, provided that two or more antral follicles were identified. This stimulation regimen, which differed from the first stimulation, consisted of letrozole 2.5 mg and HMG (225 IU/day), which were both started from the day of retrieval until the second triggering of ovulation. For both the first and second stimulations, final oocyte maturation was induced with triptorelin when follicular maturation was reached.

The first and second stimulations provided a similar number of oocytes and embryos. From the double stimulation, more than half of patients produced one to six viable embryos cryopreserved for later embryo transfer cycles with good implantation and pregnancy rate, indicating that embryos derived from double stimulation have similar development potential (*Kuang, 2014*).

Moffat et al. (2014) commented that their preliminary data appeared to confirm Kuang's findings: a second ovarian stimulation started immediately

after a first oocyte retrieval provided as many oocytes and blastocysts as obtained in the first ovarian stimulation. In this experience, both the first and second stimulations used a classical antagonist protocol with 300 IU of FSH per day, cetrorelix 0.25 mg starting on ovarian stimulation day 6 and GnRH trigger (triptorelin 0.2 mg) when follicular maturation was reached. The first and second stimulations provided a similar number of oocytes, zygotes and blastocysts, doubling the final blastocyst yield, compared with a classical single ovarian stimulation assisted reproductive technology cycle (*Moffat, 2014*).

Ubaldi et al. (2016) recently confirmed that both follicular phase and consecutive luteal phase stimulation resulted in a similar number of oocytes and embryos. Patients with reduced ovarian reserve started follicular stimulation on day 2 of the menstrual cycle with rFSH and 75 IU of recombinant LH. Daily administration of a GnRH antagonist was started when the leading follicle was 13–14 mm in diameter and continued until the day of trigger of ovulation. When at least two follicles had reached 17–18 mm in diameter, ovulation was triggered. Five days after the first oocyte retrieval a second gonadotrophin stimulation was started with the same stimulation protocol. In both follicular and luteal phase stimulation, ovulation was triggered with buserelin and oocyte retrieval was performed after 35 h. No statistically significant differences were found in the number of retrieved oocytes, MII oocytes, blastocysts or euploid blastocysts between the two stimulation cycles (*Ubaldi, 2016*). The rate of euploid embryos increased from 41.9%, considering only that obtained from the first stimulation, to 69.8% with oocytes obtained from both follicular and luteal phase stimulation.

More recently, three studies confirmed that two subsequent ovarian stimulations during the same month may maximize the number of gametes obtained (*Cardoso, 2017; Liu, 2017; Tsampras, 2017*).

Cardoso et al. (2017) analysed the performance of double stimulation in 13 patients that underwent a previous unsuccessful IVF cycle. First and second stimulations were performed with the same protocol using a high dose of gonadotrophins (225 IU rFSH and 75 IU HMG) in an antagonist protocol. The second stimulation started 5 days after the first oocyte pick-up with a GnRH agonist triggering. The mean number of oocytes collected increased from 6.7 in the previous standard follicular phase-start cycle to 11.7 with DOS.

In the study by *Liu and colleagues (2017)*, 116 women aged 38 years or older were divided into four groups according to their ovarian stimulation protocols of the follicular phase, including GnRH agonist short protocol, GnRH antagonist protocol, mild stimulation protocol and progestin pituitary down-regulation protocol. The subsequent luteal phase stimulation was performed with 225 IU HMG daily within 1–3 days of oocyte retrieval. Both triggerings were done with 250 IU rHCG. The number of oocytes retrieved (5.83 ± 4.60), MII oocytes (4.73 ± 4.01) and cleaved embryos (4.00 ± 3.42) in double stimulation were increased and the cancellation rate of no available embryos reduced (37.07% versus 18.10%) significantly compared with standard follicular phase stimulation.

Double stimulation could be useful also for patients with unexpected suboptimal response in the follicular phase, due to insufficient gonadotrophin dose. If the clinician identifies a suboptimal response during ovarian monitoring, one possibility is to stop the cycle and start again with higher gonadotrophin starting dose; the other is to move on and perform a second stimulation in case of insufficient oocyte retrieval after the first stimulation.

Finally, DOS was tested in oncological patients. Patients diagnosed with a malignancy commenced a random-start antagonist stimulation protocol. The initial dose of gonadotrophin was between 150 and 450 IU of HMG. The final maturation of oocytes was triggered by 5000 IU of HCG. On the day of oocyte collection, the patients were offered the option of a second stimulation starting the same day or after a few days. The dose of gonadotrophin was the same or increased, depending on the ovarian response in the first stimulation cycle. This pilot study demonstrated an increase in number of mature oocytes retrieved with DOS for oncology patients, without delaying cancer treatment (*Tsampras, 2017*).

According to the Tsampras study, the double randomly started ovarian stimulation (referred to here as double random ovarian stimulation) is particularly interesting and beneficial for cancer patients because it allows more oocytes for fertility preservation in a short period of time compared with a conventional-start stimulation protocol (**FIGURE 3B**).

In our clinic we usually start ovarian stimulation in oncological patients regardless of the menstrual phase. If the patient with cancer presents, at the latest, at the fourth or fifth day of the menstrual cycle and no follicles are recruited, we start ovarian stimulation the same day. If the patient presents in the follicular phase with a dominant follicle larger than 14 mm we wait for spontaneous ovulation to start stimulation or GnRH agonist is administered to trigger ovulation. If the patient presents in the luteal phase ovarian stimulation is started immediately. Ovarian stimulation is performed using recombinant FSH or HMG, at a dose of at least 200 IU. On day 5–6 of the stimulation an ultrasonic evaluation of the number and size of ovarian follicles is performed. GnRH antagonist is started to prevent LH surge when the lead follicle measures over 14 mm and continues until the GnRH agonist triggers final oocyte maturation. Triggering of final oocyte maturation is performed as soon as three follicles of ≥ 17 mm diameter are present. Thirty-six hours after triggering oocyte retrieval is performed by ultrasound-guided vaginal follicle aspiration. The oocytes collected are cryopreserved by vitrification. The second ovarian stimulation starts the day after the first oocyte pick-up with the same dose and drug used in the first one. Monitoring of ovarian response in the second ovarian stimulation is more difficult due to the presence of corpora lutea. Similar to the first stimulation, antagonist is started when the lead follicle reaches 14 mm and triggering of final oocyte maturation is performed as soon as three follicles of ≥ 17 mm diameter are present by GnRH agonist. The second pick-up is more difficult than the first due to the major risk of bleeding from the corpora lutea. This second batch of oocytes is also cryopreserved by vitrification.

Although many authors use GnRH antagonist during both follicular and

luteal phase stimulation (*Cardoso, 2017; Tsampras, 2017; Ubaldi, 2016*), it could be assumed that agonist or antagonist is not necessary in the luteal phase and that endogenous progesterone alone is sufficient to block the LH surge. Some studies conducted on luteal phase stimulation or double stimulation have demonstrated that the risk of ovulation is not increased by the absence of agonists or antagonists in the luteal phase stimulation (*Li, 2016; Liu, 2017; Massin 2017; Wang, 2016a*). It is reasonable to think that during luteal phase stimulation, both in a spontaneous cycle and subsequent to a follicular phase stimulation, the use of agonist or antagonist is useless and can only raise patient discomfort, especially in oncological patients, but this point needs to be clarified. In our clinic we continue to use the GnRH antagonist during the second stimulation, while we await further scientific evidence about the efficacy of endogenous progesterone alone in preventing LH surge.

DISCUSSION

The recognition of multiple waves of follicle development during the menstrual cycle provides a new model for understanding human ovarian follicular development. These observations suggest the possibility that ovarian follicles are available for ovarian controlled stimulation throughout the menstrual cycle independently of gonadotrophin fluctuation and that the resulting oocytes are mature and competent for fertilization.

Several studies (*Li, 2016; Qin, 2016; Wang, 2016*) indicate that ovarian stimulation can be completely disconnected from the menstrual cycle with no impact on implantation rate if no fresh embryo transfer takes place.

Because of the asynchrony between endometrial receptivity and embryo development in cases of a 'non-conventional start' stimulation, all the oocytes/embryos have to be cryopreserved and transferred subsequently. The technique of vitrification has been widely employed in recent years at the expense of the more traditional approach of cryopreservation by slow cooling. Using vitrification, cryopreserved embryo transfers have the same implantation potential as fresh embryo transfers because of high survival rates of embryos, which implant at the same rate as equivalent fresh counterparts (*Edgar and Gook, 2012*).

The possibility of starting ovarian stimulation on any day of the menstrual cycle and the fact that pregnancy rates associated with vitrified-warmed embryos are comparable with those after fresh embryo transfer allow a total disarticulation among menstrual cycle, ovarian stimulation and embryo transfer. This could be a useful tool for the clinician in order to satisfy the double demands of optimizing flexibility in ovarian stimulation and improving outcomes in IVF. DOS, consisting of two successive ovarian stimulations in the follicular and ensuing luteal phase, is a viable option when insufficient ovarian response to gonadotrophins is expected. Moreover, the short overall duration of these approaches (<30 days) is valuable for cases of fertility preservation in which the largest number of oocytes is desirable. In these cases the double stimulation may be randomly started (double random ovarian stimulation).

DOS appears to be useful, but its role in all categories of patients should be investigated. Obviously, the cost of the IVF cycle with two ovarian stimulations and two oocyte retrievals is higher due to the increasing working hours of doctors and biologists, higher number of units of gonadotrophins used, increased workload for the laboratories and the cost of vitrification. Furthermore, the switch from fresh embryo transfers to vitrified-warmed embryo transfers will be an extra cost for the couple. It follows that clinicians have to carefully choose patients who would benefit from double stimulation regimens (*Maheshwari, 2018*).

In contrast, time to pregnancy, particularly in women with low ovarian reserve, could be lower because of the short time (<30 days) in which an adequate number of oocytes is retrieved. Furthermore, adherence of patients to repeated ovarian stimulation within one cycle could be higher, because patients may be less likely to abandon the IVF treatment before the embryo transfer has been performed. In fact, after a first IVF, especially if the response has been very poor, the probability of withdrawal from IVF treatment is very high (*Brandes et al., 2009*).

Certain problems with DOS remain unsolved: firstly, it is not known which is the best day to start the second stimulation. All studies reported an interval of 1–5 days without significant difference in terms of length of stimulation and number of oocytes retrieved. There is no scientific basis to choose the duration of interval between first pick-up and the start of the second ovarian stimulation. It is also not known what the ideal drug is for triggering: some authors have reported successful pick-up with the use of HCG even in the first stimulation while the majority used GnRH agonist triggering. The utility of using a higher dose of gonadotrophin in the second stimulation or an LH activity drug is still under investigation.

In summary, other studies are needed to evaluate the efficacy of these new strategies of ovarian stimulation to improve outcomes of women with poor prognosis in IVF or that need the highest numbers of oocytes or embryos in the shortest time.

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